PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference DELBE/P32303PC	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/GB2004/005462	International filing date *(day/month/year)* 23.12.2004	Priority date *(day/month/year)* 23.12.2003
International Patent Classification (IPC) or national classification and IPC C12N15/80, C12N15/67, C12N5/10		
Applicant DELTA BIOTECHNOLOGY LIMITED et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 1 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19.07.2005	07.02.2006
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Aslund, J Telephone No. +31 70 340-4393

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/005462

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-130	as originally filed

Sequence listings part of the description, Pages

1-27	as originally filed

Claims, Numbers

5-75	as originally filed
1-4	filed with telefax on 06.01.2006

Drawings, Sheets

1/63-63/63	as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-75
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-75
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-75
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Re Item V.

Reference is made to the following documents:

D1: MARTZEN MARK R ET AL: "A biochemical genomics approach for identifying genes by the activity of their products" SCIENCE (WASHINGTON D C), vol. 286, no. 5442, 5 November 1999 (1999-11-05), pages 1153-1155, XP002325596 ISSN: 0036-8075

D2: "pYEX4T-1 Vector Information" 1998, CLONTECH CATALOG #6196-1 , XP002325601

D3: PAREKH RAJESH N ET AL: "Expression level tuning for optimal heterologous protein secretion in Saccharomyces cerevisiae" BIOTECHNOLOGY PROGRESS, vol. 13, no. 2, 1997, pages 117-122, XP002325597 ISSN: 8756-7938

D4: BAO W-G ET AL: "Secretion of human proteins from yeast: stimulation by duplication of polyubiquitin and protein disulfide isomerase genes in Kluyveromyces lactis" GENE: AN INTERNATIONAL JOURNAL ON GENES AND GENOMES, ELSEVIER SCIENCE PUBLISHERS, BARKING, GB, vol. 272, no. 1-2, 11 July 2001 (2001-07-11), pages 103-110, XP004274844 ISSN: 0378-1119

Inventive step - Article 33(3) PCT

The application concerns co-expression of a target protein and a chaperone from a 2-micron plasmid. The application states that a technical prejudice in the prior art with regard to expression of proteins from 2 micron plasmids has been overcome. The application cites (pages 3-5) documents such as D3, D4 which state that expression from 2-micron constructs is less efficient than from constructs integrated on the chromosome. Said documents speculate that this is due to overloading of the secretory machinery of the cell including overloading of chaperone functions of the secretory pathway.

Regarding expression of cytosolic target proteins, D4 provides an example where expression of a ubiqutin from a 2-micron plasmid is toxic - an effect which is overcome by chromosomal integration of the construct. On the other hand D1 shows expression on a genomewide basis of proteins from a 2 micron plasmid (see D2).

However, there is no teaching in the prior art that would prompt a person to attempt co-

expression of a chaperone and a target protein from a 2-micron plasmid. Should a person skilled in the art want to test the effect of co-expression of a chaperone along with a target protein, the approach would be conservative. I.e, in view of D3, D4, a person skilled in the art would be discouraged to include a reading frame for a chaperone on the same 2-micron plasmid as the target protein and instead opt the safer approach, namely chromosomal integration of the chaperone co-expression construct.

CLAIMS

1. A method for producing non-2μm-family plasmid protein comprising:

(a) providing a host cell comprising a 2μm-family plasmid, the plasmid comprising a gene encoding protein comprising the sequence of a chaperone protein and a gene encoding a non-2μm-family plasmid protein;

(b) culturing the host cell in a culture medium under conditions that allow the co-expression of the gene encoding protein comprising the sequence of the chaperone protein and the gene encoding a non-2μm-family plasmid protein; and

(c) purifying the thus expressed non-2μm-family plasmid protein from the cultured host cell or the culture medium.;

2. The method of Claim 1 further comprising the step of formulating the purified non-2μm-family plasmid protein with a carrier or diluent and optionally presenting the thus formulated protein in a unit dosage form.

3. Use of a 2μm-family plasmid as an expression vector to increase the production of a fungal (preferably yeast) or vertebrate non-2μm-family plasmid protein by providing a gene encoding the non-2μm-family plasmid protein and a gene encoding a chaperone protein on the same 2μm-family plasmid.

4. A 2μm-family plasmid comprising a gene encoding a protein comprising the sequence of a chaperone protein and a gene encoding a non-2μm-family plasmid protein, wherein if the plasmid is based on the 2μm plasmid then it is a disintegration vector.